[0034] The vector, or a derivative clone of interest (as described above), can also be introduced into an *Agrobacterium*-transformable non-plant eukaryotic cell. *Agrobacterium*-transformable non-plant eukaryotic cells include yeast cells such as *Saccharomyces cerevisiae, Kluyveromyces lactis;* filamentous fungi such as *Aspergillus awamori, Aspergillus niger, Fusarium venenatum, Trichoderma reesei, Colletotrichum gloeosporioides, Neurospora crass;* and the mushroom *Agaricus bisporos.* Certain hosts can be transformed by co-culturing with Agrobacterium tumefaciens strains containing the vector. The methodology is well known in the art (Groot et al., 1998; Gouka et al., 1999). Those cells containing the T-DNA are then screened for the expression of the desired gene product.

[0035] Alternatively, the vector, or a derivative clone of interest can also be introduced into a non-plant eukaryotic host cell using methods well known in the art including, for example, electroporation, nuclear particle bombardment etc. Those cells containing the vector DNA are then screened for the desired gene product. If necessary, the vector can be modified using known techniques to facilitate DNA expression in the desired eukaryotic host cells.

[0036] As used throughout this application, electroporation is a transformation method in which, generally, a high concentration of vector DNA (containing heterologous DNA) is added to a suspension of host cell protoplasts or bacterial cells and the mixture is shocked with an electrical field of 200 to 600 V/cm. Following electroporation, transformed cells are identified by growth on appropriate medium containing a selective agent (Mozo and Hooykaas, 1991).

[0037] As also used throughout this application, particle bombardment (also know as biolistic transformation) of the host cell can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in

U.S. Patent Nos. 4,945,050; 5,036,006; and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into cells.

[0038] Thus, the methods of the present invention can be used to transform a number of diverse host cells in a variety of ways. Specifically, heterologous DNA encoding the desired gene product, or a library of DNAs can be inserted into the unique restriction endonuclease cleavage site of the vector, e.g. BIBAC. The vector, containing the heterologous DNA, is used to transform a bacterial host cell e.g., *Escherichia coli*, or *Agrobacterium tumefaciens*. The transformed bacterial cells can be screened for expression of the desired gene product. The vector can also then be used to transform non-plant host cells including yeast; prokaryotic; mammalian, reptile, bird, etc. Preferably, the host cell is a yeast or filamentous fungus and the transformation is *Agrobacterium*-mediated. The cells containing the vector can then be screened for expression of the desired gene product. The introduction of the heterologous DNA into the host cell allows the production of the gene product encoded by the heterologous DNA when the DNA is expressed in the cell.

[0039] Alternatively, a desired gene product can be produced in a non-plant eukaryotic cell by transforming a cell directly with the vector (having heterologous DNA encoding the gene product inserted into the unique restriction endonuclease cleavage site), such as electroporation.

[0040] With such methods in mind, the method of the present invention can be used to isolate genes, including gene clusters, by their phenotype. There are many examples from prokaryotic organisms and some examples from eukaryotic organisms, in which genes involved in the same biosynthetic pathway are genetically and physically linked (Lawrence, 1999). For example, a 25-kb DNA region from *Pseudomonas syringae* confers upon *E. coli* the ability to induce hypersensitive response on tobacco – though normally *E. coli* would not secrete the appropriate signals to cause this response (Alfano *et al.*, 1997). Enzymes which can catalyze the degradation of nicotine are located on a 160-kb plasmid in *Arthrobacter nicotinovorans* (Shenk and Decker, 1999).

[0041] There are also examples of clusters of genes involved in particular biochemical pathways in plants. Pectin methylesterase genes are clustered in *Arabidopsis* (Richard *et al.*, 1996), two tomato alcohol dehydrogenase genes are closely linked (Ingersoll *et al.*, 1994), and the *Brassica* self-incompatibility locus spans several hundred kb and contains a number of linked and co-adapted genes (Boyes *et al.*, 1997).

[0042] In another embodiment, varying size segments (small-to-large) of DNAs or cDNAs from an organism is cloned into a host bacterium and then the resulting strains screened for production of a product of interest. The host bacterium can be grown either on standard media or fed potential precursors of useful products. For example, antibiotics, novel pigments, enzymes active in temperature extremes, etc.

[0043] As used throughout this application, transformation encompasses either transient or stable transformation. In transient transformation, heterologous DNA is introduced into a host cell without being incorporated into the DNA of the host cell (incorporation being a stable transformation).